

Version with markings to show changes made

In the specification:

Paragraph beginning at page 3, line 32, has been amended as follows:

Figure 2 is a compilation of graphical representations of data which indicate that [PS] pregnenolone sulfate (PS) inhibits [AMPA] α -amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) and kainate receptor function. Figures [1(A)]2(A) through [1(D)]2D are representative traces showing the inhibitory effect of 100 μ M PS on kainate-induced currents of oocytes injected with (A) rat brain poly(A)⁺ RNA, (B) GluR1 cRNA, (C) GluR3 cRNA, (D) GluR6 cRNA. The kainate concentration used in (A)-(C) was 100 μ M, and in (D) was 10 μ M. The *solid bar* represents the period of kainate (KA) application; the *open bar* indicates the period of PS exposure. Figure [1(E)]2E is a graph of relative current for the indicated Kainate concentration. The administration of PS (open symbols) is seen to decrease maximum kainate responses of GluR1 (●, ○), GluR3 (■, □), and GluR6 (▲, Δ) receptors. Each *data point* represents the mean of three experiments. *Error bars* represent standard error. *Smooth curve* was determined by nonlinear regression using the logistic equation applied to pooled data. Fitted parameters are (GluR1) $I_{\max}=1.0$, $EC_{50}=27 \mu\text{M}$, $n_H=1.54$; (GluR1 + PS) $I_{\max}=0.17$, $EC_{50}=23 \mu\text{M}$, $n_H=0.9$; (GluR3) $I_{\max}=1.15$, $EC_{50}=27 \mu\text{M}$, $n_H=1.44$; (GluR3 + PS) $I_{\max}=0.33$, $EC_{50}=32 \mu\text{M}$, $n_H=1.93$; (GluR6) $I_{\max}=1.0$, $EC_{50}=550 \text{ nM}$, $n_H=1.1$; (GluR6 + PS) $I_{\max}=0.69$, $EC_{50}=570 \text{ nM}$, $n_H=1.2$. Figure [1(F)]2F is a graph of data showing the concentration dependence of PS inhibition of recombinant GluR1 (○), GluR3 (□), and GluR6 (▲) receptors. Results are expressed as percentage change in the peak 100 μ M (GluR1 and GluR3) or 10 μ M (GluR6) kainate-induced current in the presence of PS. Each *data point* is the mean of three experiments; *error bars* indicate S.E.M. For GluR1 and GluR3, *smooth curves* are derived from fits to the Michaelis-Menten equation, as fits to the logistic equation yielded Hill coefficients close to 1, with no significant improvement in sum of squares (F -test, $P > 0.05$). Fitted parameters are (GluR1) $EC_{50}=43 \mu\text{M}$, $E_{\max} = -99\%$; (GluR3) $EC_{50}=12 \mu\text{M}$, $E_{\max} = -90\%$. For GluR6, the smooth curve is derived from a fit to the logistic equation, as Michaelis-Menten fits were significantly poorer (F -test, $P < 0.05$). Maximum inhibition was constrained to 100%, as an

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unconstrained fit yielded an extrapolated maximum inhibition >100%. Fitted parameters are $EC_{50}=80 \mu\text{M}$, $n_H=0.29$.

Paragraph beginning at page 5, line 4, has been amended as follows:

Figure 3 is a compilation of graphical representations of data which indicate that neuroactive steroids modulate NMDA responses of oocytes injected with specific NMDA receptor subunits. Figure [2(A)]3(A) indicates the potentiation of the $100 \mu\text{M}$ NMDA response by PS in oocytes injected with NR1₁₀₀ + NR2A cRNA. The *solid bar* indicates the period of NMDA exposure; the *open bar* indicates the period of PS exposure. Figure [2(B)]3(B) indicates inhibition of the $100 \mu\text{M}$ NMDA response by $3\alpha 5\beta\text{S}$ in oocytes injected with NR1₁₀₀ + NR2A cRNA. The *solid bar* indicates the period of NMDA exposure; the *shaded bar* indicates the period of $3\alpha 5\beta\text{S}$ exposure. Figure [2(C)]3(C) indicates modulation of agonist efficacy by PS and $3\alpha 5\beta\text{S}$ in oocytes injected with NR1₁₀₀ + NR2A cRNA. PS ($100 \mu\text{M}$) increases the NMDA I_{max} but does not affect the EC_{50} . $3\alpha 5\beta\text{S}$ ($100 \mu\text{M}$) markedly reduces the NMDA I_{max} with little effect on EC_{50} . Peak NMDA responses are normalized to the peak $100 \mu\text{M}$ NMDA response. Each *data point* represents the mean of three experiments. *Error bars* represent standard error. *Smooth curves* are derived from fits to the logistic equation. Fitted parameters are (control) $EC_{50}=29 \mu\text{M}$, $E_{\text{max}}=1.14$, $n_H=1.43$; (+PS) $EC_{50}=30 \mu\text{M}$, $E_{\text{max}}=3.21$, $n_H=1.54$; (+ $3\alpha 5\beta\text{S}$) $EC_{50}=15 \mu\text{M}$, $E_{\text{max}}=0.35$, $n_H=1.66$. Figure [2(D)]3(D) is a graph indicating the concentration dependence of steroid modulation of the NMDA response of oocytes injected with NR1₁₀₀ + NR2A cRNA. NMDA ($100 \mu\text{M}$) and the indicated concentration of PS (●), $3\beta 5\beta\text{S}$ (Δ), or $3\alpha 5\beta\text{S}$ (□) were applied simultaneously for 10 s. The peak NMDA-induced current is expressed relative to the average of control NMDA responses determined before application of steroid and after steroid washout. *Points* indicate mean of 6 (PS and $3\alpha 5\beta\text{S}$), and 4 ($3\beta 5\beta\text{S}$), experiments. *Error bars* indicate S.E.M. Smooth curves are derived from fits to the Michaelis-Menten equation, as fits to the logistic equation yielded Hill coefficients close to 1, with no significant improvement in sum of squares (*F*-test, $P > 0.05$). Fitted parameters are (for PS) $EC_{50}=32 \mu\text{M}$, $E_{\text{max}}=4.43$ (for $3\alpha 5\beta\text{S}$) $EC_{50}=41 \mu\text{M}$, $E_{\text{max}}=0.1$; (for $3\beta 5\beta\text{S}$) $EC_{50}=79 \mu\text{M}$, $E_{\text{max}}=0.26$. (E) Concentration dependence for PS enhancement (●) and $3\alpha 5\beta\text{S}$ (Δ) and $3\beta 5\beta\text{S}$ (□) inhibition of the NMDA response of oocytes